Cancer Incidence in Denmark Following Exposure to Poliovirus Vaccine Contaminated With Simian Virus 40

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Background: Early poliovirus vaccines were accidentally contaminated with simian virus 40 (SV40). In Denmark, poliovirus vaccine was administered to most children from 1955 through 1961. SV40 DNA sequences have been detected in several human malignancies, including mesothelioma, ependymoma, choroid plexus tumors, and non-Hodgkin's lymphoma. To clarify whether SV40 infection increases risk of these cancers or of cancers arising in children, we examined cancer incidence in three Danish birth cohorts. *Methods:* Population-based cancer incidence data from 1943 through 1997 were obtained from the Danish Cancer Registry. The relationship between exposure to SV40-contaminated vaccine and cancer incidence was evaluated by examining incidence in birth cohorts that differed in exposure to SV40-contaminated vaccine. In addition, cancer incidence was examined in children who were 0–4 years of age before, during, and after the period of vaccine contamination. Incidence was compared using Poisson regression, adjusting for age differences. All statistical tests were two-sided. *Results:* After 69.5 million person-years of follow-up, individuals exposed to SV40-contaminated poliovirus vaccine as infants (i.e., born 1955–1961) or children (i.e., born 1946–1952) had lower overall cancer risk (age-adjusted relative risk [RR] = 0.86, 95% confidence interval [CI] = 0.81 to 0.91 and RR = 0.79, 95% CI = 0.75 to 0.84, respectively; $P<.001$ for both) than unexposed individuals (i.e., born 1964–1970, after the vaccine was cleared of SV40 contamination). Specifically, SV40 exposure was not associated with increased incidence of mesothelioma, ependymoma, choroid plexus tumor, or non-Hodgkin's lymphoma. After 19.5 million person-years of follow-up, incidence of all cancers combined, of intracranial tumors, and of leukemia among children aged 0–4 years was also not associated with SV40 exposure. Ependymoma incidence was higher during the exposed period than during the unexposed period (RR = 2.59, 95% CI = 1.36 to 4.92; $P = .004$ versus the period before contamination); however, incidence peaked in 1969, after the vaccine was cleared of SV40. *Conclusion:* Exposure to SV40-contaminated poliovirus vaccine in Denmark was not associated with increased cancer incidence. [J Natl Cancer Inst 2003;95:532–9]

Simian virus 40 (SV40), a macaque polyomavirus, was an accidental contaminant of early poliovirus vaccines *(1)*. This contamination arose because, before the 1960 discovery of SV40 *(2),* poliovirus vaccines were produced in monkey kidney tissue harboring SV40, and manufacturing procedures did not completely inactivate the virus *(1,3)*. These poliovirus vaccines were administered to tens of millions of people worldwide. This widespread exposure raised public health concern, because the SV40 genome encodes a protein (i.e., T antigen) that inactivates the cellular tumor suppressor proteins p53 and pRb *(4),* and SV40 has been shown to cause malignancies (e.g., mesothelioma, ependymoma, osteosarcoma, leukemia, and lymphoma) in rodents *(5–8)*. Some researchers have identified SV40 DNA sequences in similar human tumors, most recently non-Hodgkin's lymphoma *(9–15)*.

In Denmark, poliovirus vaccine was first administered in April 1955, a few weeks after vaccination campaigns began in the United States. Because of the urgent need to combat the poliomyelitis epidemic, Danish public health officials mounted a concerted campaign to administer poliovirus vaccine to a large

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proportion of the Danish population (i.e., approximately 4.6 million people), especially children and young adults (16). By September 1956, 86% of children aged 9–23 months, 98% of children aged 2–6 years, and 99% of children aged 7–14 years had received one or more doses of poliovirus vaccine (17). With continued vigorous public health efforts directed especially at children, Denmark maintained a high level of vaccination throughout the late 1950s and early 1960s. As of April 1962, 90% of children aged 9–23 months and 84%–100% of children aged 2–19 years had received at least one dose of poliovirus vaccine (18). Notably, as we describe below, Danish poliovirus vaccine used from 1955 through much of 1962 was widely contaminated with SV40.

In the present investigation, we studied the association between SV40 and risk of cancer by examining cancer incidence in Danish cohorts with varying exposure to SV40-contaminated poliovirus vaccine. We documented the extent of SV40 contamination of the poliovirus vaccine through a review of records at the Statens Serum Institut. We focused on cancer outcomes in adults (mesothelioma, brain tumors, osteosarcoma and other bone tumors, non-Hodgkin's lymphoma, leukemia, and testicular carcinoma) that were identified in previous human or laboratory studies as possibly linked with SV40 infection. Because SV40 is most tumorigenic in newborn laboratory animals (19), we expected that, for cancers caused by SV40, incidence would be especially high in individuals exposed as infants, intermediate in those exposed as children, and lowest in those who were unexposed. We also examined cancers arising in children, to determine whether the risk of childhood cancers was elevated following exposure to SV40-contaminated poliovirus vaccine.

METHODS

Determination of Extent of SV40 Contamination of Danish Poliovirus Vaccine

In Denmark, early inactivated poliovirus vaccine was produced by a single manufacturer (Statens Serum Institut, Copenhagen) using poliovirus seeds from Sweden (type 1) and the United States (types 2 and 3) (20). Vaccine virus was grown using a monolayer method that pooled kidney cells from dozens of monkeys, which greatly increased the likelihood of SV40 contamination of the final product (1,21).

To assess the scope of SV40 contamination of early poliovirus vaccine in Denmark, we reviewed internal documents from the Statens Serum Institut. These documents describe results from tests for SV40, which were conducted in 1961 on previously released lots of poliovirus vaccine. We reviewed additional internal documents describing results from the 1962 testing of serum samples from vaccinated individuals for evidence of SV40 exposure.

In January 1962, with contemporaneous evidence for SV40 contamination of the Danish poliovirus vaccine, production of the vaccine was halted. However, given the vaccine's 6-month shelf-life, previously released vaccine could have been used throughout much of 1962. Changed production techniques eliminated SV40 from vaccines released in 1963 and thereafter (Statens Serum Institut, internal documents).

Cancer Incidence

Data on cancer counts and population size in 1-year age and calendar-year intervals were obtained from the Danish Cancer Registry from 1943 through 1997. For ascertainment of cancers grouped by site over this time period, we used two-digit group codes according to a national modification of the seventh revision of the International Classification of Diseases (ICD-7*) (22). Because of changes in ICD coding in the Danish Cancer Registry, we used different definitions for identification of specific cancer types in the time periods 1943–1977 and 1978–1997. Specifically, for the time period 1943–1977, we used individual four-digit ICD-7* codes, which incorporate information about histology, topography (i.e., site of tumor), and behavior (i.e., malignant versus other). To identify specific cancer types for the time period 1978–1997, we used topography (T) and histology (M) codes, according to the first edition of the International Classification of Diseases for Oncology (ICD-O1) (23), and we restricted tumor behavior to code 3 (i.e., malignant). We studied the following cancer outcomes: all cancers combined, mesothelioma (ICD-7* codes 1581, 4622, and 4644; ICD-O1 codes T1589, T1639, T1641, and T1878, each with M905), all brain and nervous system tumors combined (ICD-7* group code 55), all bone tumors combined (ICD-7* group code 58), osteosarcoma (data available only from 1978 onward, ICD-O1 codes T170 and M918–M919), leukemia (ICD-7* group code 67), non-Hodgkin's lymphoma (ICD-7* group code 64), and testicular carcinoma (ICD-7* group code 45). For ependymoma and choroid plexus tumor, we used national incidence data derived from the Danish Cancer Registry and from a previous review of medical files at all neurosurgical departments in Denmark (24,25). Data from these combined sources were limited to children aged 0–14 years and to the time period from 1943 through 1984. These data were also used for an analysis of all intracranial tumors combined in children (24,25).

Statistical Analysis

We used two complementary approaches to examine cancer incidence as a function of exposure to SV40-contaminated poliovirus vaccine. In the first approach, we compared long-term cancer incidence in subgroups of individuals born from 1946 through 1970: the 1946–1952 birth cohort, almost all of whom were vaccinated in 1955 when the vaccine first became available (i.e., exposed as children at ages 2–9 years); the 1955–1961 birth cohort, almost all of whom were vaccinated at approximately 9 months of age or soon thereafter (i.e., exposed as infants); and the 1964–1970 birth cohort, who were born after the vaccines were cleared of SV40 contamination (i.e., unexposed individuals). Individuals born from 1953 through 1954 and from 1962 through 1963 were excluded from analysis to provide a clear separation between birth cohorts. Follow-up was from 1955 or the year of birth, whichever came later, through 1997. Follow-up was shorter for osteosarcoma, ependymoma, and choroid plexus tumors, as noted above.

Age strongly affects cancer incidence, and the three birth cohorts in our study attained different age ranges during follow-up. Therefore, for descriptive purposes, we present age-standardized incidence in the three birth cohorts for the various cancer outcomes (i.e., specific cancer types evaluated). The reference population for these incidence rates was the 1980 Danish population, restricted to ages 5–29 years to limit consideration to the overlapping age range attained by all three cohorts. For ependymoma, choroid plexus tumors, and osteosarcoma, where shorter follow-up resulted in incomplete coverage of the 5- to 29-year age window, we present crude incidence instead.

To formally test for differences in cancer incidence across birth cohorts, we used Poisson regression *(26),* controlling for age using a regression spline-based approach *(27).* Specifically, we modeled $\ln(n_{a,c}) = \ln(p_{a,c}) + f_1(a) + f_2(c)$, where $n_{a,c}$ was the number of cancers of a particular type in persons of age a in birth cohort c; $p_{a,c}$ was the corresponding number of person-years; f_1 was a function of age (i.e., linear, quadratic, or cubic polynomial or spline with 2–10 segments); and f_2 was a three-level variable indicating birth cohort. The fitted values and associated confidence intervals (CIs) for f_2 provide age-adjusted relative risks (RRs) for comparing incidence across birth cohorts. Incorporating a spline in the regression models allowed us to use all of the available data across ages to optimally model the observed age-specific incidence rates, thus increasing the statistical power for comparing cancer incidence across birth cohorts. We selected the best-fitting model based on the Bayesian Information Criterion (BIC) statistic *(28).* Model fits were checked graphically using plots of standardized residuals against age. Only one model (for all cancers combined) exhibited overdispersion, and CIs were widened accordingly *(26).*

In the second approach, we examined the incidence over time of several cancer outcomes (i.e., all cancers combined, intracranial tumors, leukemia) among children aged 0–4 years. This approach allowed us to look at short-term cancer risk associated with early-life exposure to SV40-contaminated poliovirus vaccine. In this analysis, cancer outcomes and follow-up time in these young children were classified by calendar year and, secondarily, by the child's year of birth. All cancer outcomes and person-years in children who were 0–4 years old during 1943–1954 were classified as occurring in an "unexposed" period, because they occurred before the introduction of SV40-contaminated poliovirus vaccine. Cancer outcomes and person-years in children who were 0–4 years old during 1955–1962 were classified as occurring in the "exposed" period, that is, the period when poliovirus vaccines were contaminated. For the period 1963–1966, we also included in the exposed period those cancer outcomes and person-years occurring in children who were born before 1963 (i.e., exposed to contaminated vaccine)—that is, cancer outcomes and person-years occurring in 4-year-old children in 1963–1966, cancer outcomes and person-years occurring in 3-year-old children in 1963–1965, and so on. All other cancer outcomes and person-years in children who were 0–4 years of age were classified as occurring in the "post-exposed" period because they occurred in children born after the poliovirus vaccine was cleared of SV40 contamination.

On the basis of these cancer counts and person-years, we calculated incidence among children aged 0–4 years for each of the three exposure periods. Under the assumption that SV40 causes childhood cancer, one would expect an increase in incidence from the unexposed to the exposed period, followed by a decrease in incidence in the post-exposed period. We used Poisson regression to compare incidence across categories of person-years. The model for all cancers combined again exhibited overdispersion, and CIs for the RRs were again widened accordingly *(26).* In addition, we smoothed the calendar-year-specific incidence for ependymoma among children aged 0–4 years to better model temporal changes in the incidence of this rare cancer. Specifically, we fitted a series of low-order polynomials under a Poisson model, selecting the best-fitting curve with likelihood ratio statistics *(26).*

All statistical analyses were conducted with MATLAB (version 6.1; Mathworks, Natick, MA). All CIs and significance tests were two-sided.

RESULTS

Extent of SV40 Contamination of Danish Poliovirus Vaccine

To determine the scope of SV40 contamination of Danish poliovirus vaccine, we analyzed original documents from the Statens Serum Institut. Internal documents from late 1961 and early 1962 describe the evaluation of lots of formalin-inactivated, trivalent poliovirus vaccine previously released in 1957–1961. Specifically, in October 1961, 0.2 mL of vaccine from each of six lots was inoculated onto monolayer cultures of *Cercopithecus* monkey kidney cells and observed over two passages (i.e., 21 and 14 days, ±1 day, respectively) for an SV40-specific cytopathic effect. Two lots of vaccine (released in 1957 and 1958) were SV40-positive in the first passage, while three additional lots (released in 1959 and 1961) were SV40-positive in the second passage. Further testing in November 1961 evaluated three already-tested lots, including the previously negative lot, and three additional lots. In this testing, a larger inoculum (35 mL) was added to *Cercopithecus* kidney-cell monolayer cultures, for passages of 21 and 14 days (±1 day). Three lots of vaccine (released in 1961) were SV40-positive in the first passage and the remaining three lots (also released in 1961) were SV40-positive in the second passage, including the previously negative lot.

As detailed in additional documents from the Statens Serum Institut (dated March 1962), stored sera from three employees involved in vaccine production were analyzed for SV40-neutralizing antibodies. All three employees lacked detectable SV40 antibodies in prevaccination sera, but after their first receipt of poliovirus vaccine in early 1955, each developed medium- or high-titer SV40-neutralizing antibodies.

The demonstration of infectious SV40 in all examined lots of vaccine released from 1957 through 1961 and the detection of SV40 seroconversion in individuals vaccinated in 1955 indicate that most, if not all, Danish poliovirus vaccine released from 1955 through 1961 contained live SV40. Some lots of vaccine had SV40 that was detectable in a single passage from a small inoculum, suggesting a high titer of live virus.

Cancer Incidence in SV40-Exposed and Unexposed Birth Cohorts

We examined long-term cancer incidence in three birth cohorts with varying types of exposure to SV40-contaminated Danish poliovirus vaccine: individuals exposed as children or infants (1946–1952 and 1955–1961 birth cohorts, respectively) and individuals who were not exposed (1964–1970 birth cohort). Fig. 1 shows the observed and fitted estimates of age-specific incidence for selected cancer outcomes. Overall, the fitted incidence estimates correspond closely to the observed incidence rates. Incidence of all cancers combined (Fig. 1, A), all brain and nervous system tumors combined (Fig. 1, C), and non-Hodgkin's lymphoma (Fig. 1, F) increased steadily with age. Mesothelioma was rare (i.e., 57 case patients), especially among individuals younger than 40 years, for whom most data were collected (Fig. 1, B). The age-specific incidence patterns for bone tumors (Fig. 1, D) and osteosarcoma (Fig. 1, E) were similar, with a peak in incidence at age 16–17 years. Data on

Fig. 1. Age-specific cancer incidence for three Danish birth cohorts with varying exposure to simian virus 40 (SV40)-contaminated poliovirus vaccine. Incidence data are shown for all cancers combined (**A**), for mesothelioma (**B**), for all brain and nervous system tumors combined (**C**), for all bone tumors combined (**D**), for osteosarcoma (**E**), and for non-Hodgkin's lymphoma (**F**). The three birth cohorts are 1946–1952 (exposed to SV40-contaminated poliovirus vaccine as children, **orange line**), 1955–1961 (exposed to SV40-contaminated poliovirus vaccine as infants, **black line**), and 1964–1970 (unexposed individuals, **blue line**). Both observed incidence (**thin lines**) and fitted estimates derived from a Poisson model (**thick lines**) are shown. Incidence is per 100 000 person-years (vertical scales vary).
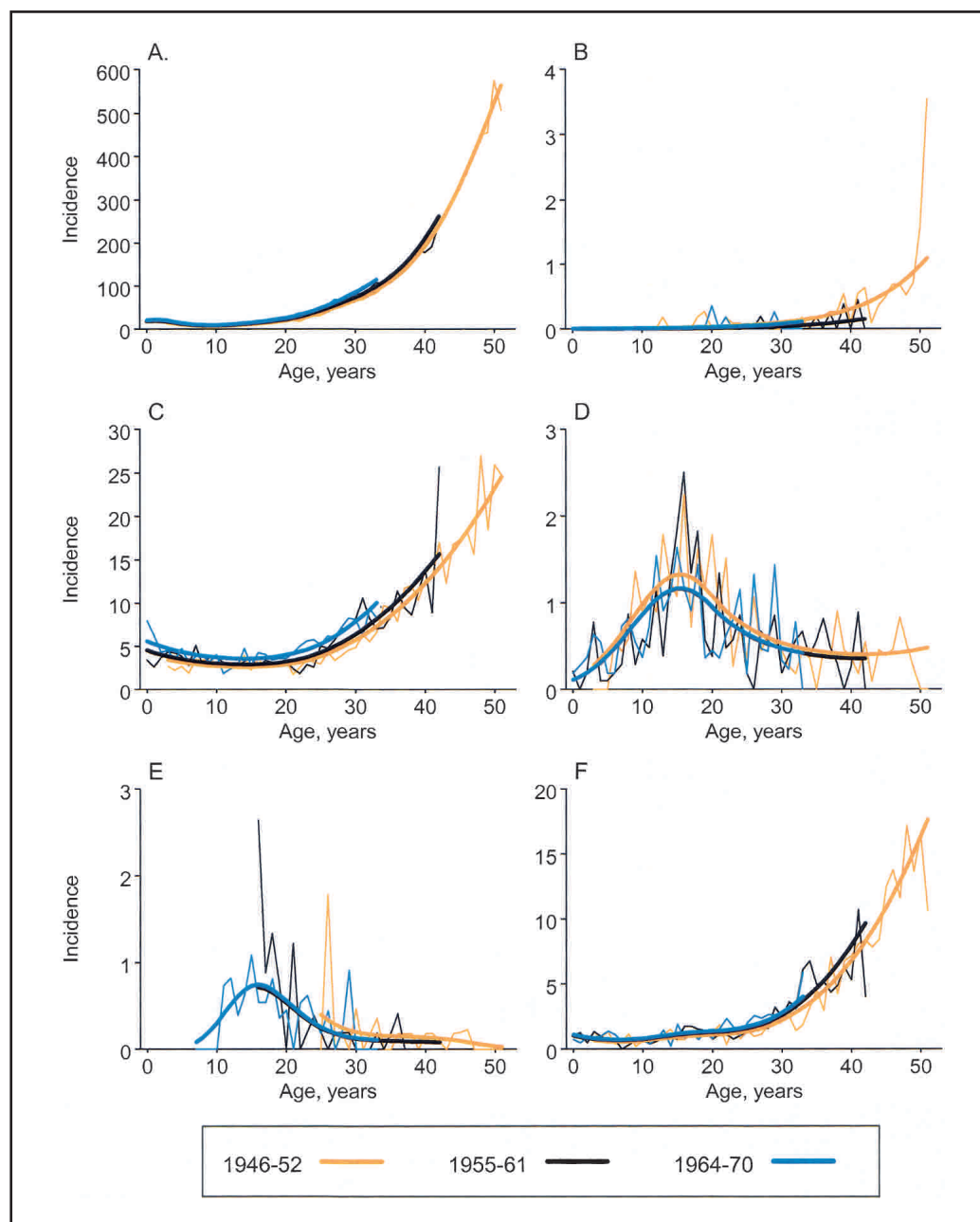
osteosarcoma were not available before 1978. Thus, the overlap in ages across the three cohorts was reduced, and only the 1964–1970 birth cohort provided data on osteosarcoma incidence in children (Fig. 1, E). Nevertheless, among individuals who were less than 25 years old during the period when osteosarcoma data were available, osteosarcoma comprised 60% of bone tumors. Therefore, it is reasonable to assume that the fitted curves for osteosarcoma, which have the same shape as those for all bone tumors, accurately capture the peak in age-specific incidence.

Table 1 presents the incidence of each cancer outcome and age-adjusted RRs, which are derived from the regression models illustrated in Fig. 1. For each cancer, age-adjusted RRs were not statistically significantly elevated for either exposed cohort (i.e., the 1946–1952 and the 1955–1961 cohorts). In fact, compared with the unexposed cohort, the two exposed cohorts each had statistically significantly lower risk for all cancers combined (*P*<.001 for both the 1946–1952 and 1955–1961 cohorts) and all

brain and nervous system tumors combined (*P*<.001 for both cohorts). Similarly, when the two exposed cohorts were considered together, the combined cohort did not have a higher cancer risk over that of the unexposed cohort for any cancer outcome (Table 1). In addition, when males and females were analyzed separately, none of the cancers studied had statistically significantly higher incidence in the exposed cohorts than in the unexposed cohort (data not shown).

There was a slight excess in the incidence of ependymoma in the cohort exposed as infants compared with the unexposed cohort (incidence = 0.51 versus 0.41 per 100 000 person-years, respectively); however, the corresponding age-adjusted RR was not statistically significantly elevated (RR = 1.25, 95% CI = 0.79 to 1.98; *P* = .35). Similarly, ependymoma risk was not statistically significantly elevated in the cohort exposed as children compared with the unexposed cohort (RR = 1.18, 95% CI = 0.66 to 2.11; *P* = .59) or when the two exposed cohorts were

Table 1. Cancer incidence in birth cohorts with varying exposure to simian virus 40-contaminated poliovirus vaccine

Cancer outcome	Incidence per 100 000 person-years, (n)*			Age-adjusted relative risk (95% confidence intervals) P value†		
	1946–1952 (Exposed as child)	1955–1961 (Exposed as infant)	1964–1970 (Unexposed)	1946–1952 vs. 1964–1970 (Effect of exposure as child)	1955–1961 vs. 1964–1970 (Effect of exposure as infant)	1946–1952 and 1955–1961 vs. 1964–1970 (Effect of exposure as infant or child)
All cancers	20.9 (27 350)	24.4 (11 105)	27.4 (5518)	0.79 (0.75 to 0.84) <.001	0.86 (0.81 to 0.91) <.001	0.82 (0.78 to 0.88) <.001
Mesothelioma	0.04 (47)	0.01 (6)	0.03 (4)	1.23 (0.37 to 4.07) .74	0.48 (0.12 to 1.83) .28	0.82 (0.26 to 2.61) .74
Brain and nervous system tumors	3.12 (672)	3.68 (630)	4.37 (488)	0.73 (0.66 to 0.81) <.001	0.81 (0.74 to 0.90) <.001	0.78 (0.71 to 0.85) <.001
Ependymoma‡	0.35 (21)	0.51 (45)	0.41 (38)	1.18 (0.66 to 2.11) .59	1.25 (0.79 to 1.98) .35	1.23 (0.80 to 1.88) .35
Choroid plexus tumor‡	0.02 (1)	0.03 (3)	0.11 (10)	0.47 (0.05 to 4.52) .51	0.26 (0.06 to 1.24) .09	0.30 (0.08 to 1.16) .08
All bone tumors	0.97 (192)	0.80 (153)	0.81 (142)	1.13 (0.89 to 1.45) .31	1.00 (0.78 to 1.28) .98	1.06 (0.86 to 1.32) .58
Osteosarcoma§	0.13 (17)	0.22 (26)	0.45 (57)	1.58 (0.61 to 4.05) .34	0.95 (0.53 to 1.71) .88	0.93 (0.52 to 1.65) .80
Leukemia	2.57 (859)	2.45 (711)	2.40 (626)	1.09 (0.96 to 1.25) .18	0.96 (0.85 to 1.08) .46	0.99 (0.89 to 1.10) .82
Non-Hodgkin's lymphoma	1.12 (973)	1.11 (480)	1.34 (250)	0.79 (0.66 to 0.95) .01	0.93 (0.78 to 1.11) .41	0.89 (0.75 to 1.04) .15
Testicular carcinoma	5.69 (1472)	8.26 (1416)	8.62 (806)	0.67 (0.60 to 0.74) <.001	0.93 (0.84 to 1.02) .13	0.80 (0.73 to 0.88) <.001

*Total person-years of follow-up were 26.7 million for the 1946–1952 cohort, 23.5 million for the 1955–1961 cohort, and 19.4 million for the 1964–1970 cohort. Age-standardized incidence rates are shown (standardized to the 1980 Danish population, aged 5–29 years) for all malignancies except ependymoma, choroid plexus tumor, and osteosarcoma. For those cancer outcomes, shorter follow-up time resulted in incomplete coverage of the 5- to 29-year age window; thus, crude incidence rates are presented.

†P value was determined using Poisson regression.

‡Data on histologically verified ependymomas and choroid plexus tumors were limited to individuals 0–14 years old.

§Data on osteosarcoma were available beginning in 1978.

considered together, again in comparison with the unexposed cohort (RR = 1.23, 95% CI = 0.80 to 1.88; P = .35).

Cancer Incidence in Children Aged 0–4 Years

To examine the effect of exposure to SV40-contaminated vaccine on cancer incidence among children aged 0–4 years, we evaluated incidence data in three different exposure periods: unexposed, exposed, and post-exposed (Table 2). The incidence of all cancers combined was similar in the exposed and unexposed periods and was higher in the post-exposed period—that is, children who had never received SV40-contaminated vaccine (i.e., post-exposed) had a risk of cancer that was 1.23 (95% CI = 1.07 to 1.40) times that of children who had been exposed to SV40-contaminated vaccine. For leukemia and all intracranial

Table 2. Cancer incidence in children aged 0–4 years by exposure to simian virus 40 (SV40)-contaminated poliovirus vaccine*

Cancer outcome	Incidence per 100 000 person-years, (n)†			Relative risk (95% confidence intervals) P value‡	
	Unexposed	Exposed	Post-exposed	Exposed vs. unexposed	Post-exposed vs. exposed
All cancers	16.43 (789)	16.57 (614)	20.35 (2240)	1.01 (0.86 to 1.18) .92	1.23 (1.07 to 1.40) .003
Intracranial tumors	2.46 (118)	3.02 (112)	3.58 (253)	1.23 (0.95 to 1.59) .12	1.18 (0.95 to 1.48) .14
Ependymoma	0.29 (14)	0.76 (28)	0.72 (50)	2.59 (1.36 to 4.92) .004	0.94 (0.59 to 1.49) .78
Choroid plexus tumor	0.08 (4)	0.05 (2)	0.20 (14)	0.65 (0.12 to 3.54) .62	3.67 (0.83 to 16.15) .09
Leukemia	6.56 (315)	6.91 (256)	7.42 (817)	1.05 (0.89 to 1.24) .54	1.07 (0.93 to 1.24) .32

*With respect to SV40-contaminated poliovirus vaccine, cancer outcomes and person-years among children aged 0–4 years were classified as unexposed (calendar year before 1955), exposed (calendar years 1955–1962 or 1963–1966 for children born before 1963, i.e., children who were 4 years old in 1963–1966, children who were 3 years old in 1963–1965, and so on), or post-exposed (other cancer outcomes and person-years).

†Total follow-up time was 4.8 million person-years for the unexposed period, 3.7 million person-years for the exposed period, and 11.0 million person-years for the post-exposed period (except for intracranial tumors, where follow-up was 7.1 million person-years in the post-exposed period because of the lack of data after 1984).

‡P value was determined using Poisson regression.

tumors combined, there were gradual, albeit not statistically significant, increases in incidence from unexposed to exposed, and from exposed to post-exposed periods (Table 2). For choroid plexus tumor, risk was lower (although not statistically significantly lower) in the exposed period than it was in the unexposed period (RR = 0.65, 95% CI = 0.12 to 3.54).

Ependymoma manifested a different incidence pattern (Table 2). Ependymoma incidence was higher in the exposed period than it was in the unexposed period (0.76 versus 0.29 per 100 000 person-years, respectively), corresponding to a statistically significantly elevated risk (RR = 2.59, 95% CI = 1.36 to 4.92; P = .004). However, incidence did not decline in the post-exposed period (0.72 per 100 000 person-years) after poliovirus vaccine had been cleared of SV40 contamination (RR = 0.94, 95% CI = 0.59 to 1.49 compared with the exposed period; P = .78). To further investigate the incidence pattern of ependymoma, we evaluated incidence by calendar year (Fig. 2). The highest single-year incidence was observed in 1964, which was after the poliovirus vaccine was cleared of SV40 contamination. Seven cases of ependymoma occurred in that year, four of which occurred in children aged 0–1 years who were too young to have received SV40-contaminated poliovirus vaccine. Because ependymoma was rare and individual-year estimates of incidence were based on small numbers of cases, we obtained smoothed estimates of calendar-year-specific ependymoma incidence rates using a family of regression polynomials. The best-fitting curve was a quadratic polynomial (Fig. 2), which had a peak in incidence in 1969 (95% CI = 1963 to 1975).

DISCUSSION

In our analysis of cancer incidence data collected from the Danish Cancer Registry over a 55-year period, cancer incidence was not associated with exposure to SV40-contaminated poliovirus vaccine. With long-term follow-up (69.5 million person-years), the exposed and unexposed cohorts had similar cancer incidence (Table 1). Indeed, overall cancer incidence in cohorts with high levels of SV40 exposure as infants or as children was lower than that in individuals born later, i.e., after SV40 was cleared from poliovirus vaccines. We specifically looked for excess cancer risk in individuals exposed to SV40 as infants because of their developmental immaturity and because of previous animal data demonstrating a strong tumorigenic effect in newborn animals (19); however, this cohort did not have a statistically significantly increased risk for any of the cancer outcomes studied.

Similarly, overall cancer incidence was not increased in young children (0–4 years of age) who were exposed to SV40-contaminated poliovirus vaccine (Table 2). The slight increases over time in the incidence of intracranial tumors [previously noted in (25)] and leukemia are less consistent with an SV40 effect than with gradual changes in other unknown exposures or with improvements in diagnostic methods. Interestingly, the incidence of choroid plexus tumors among these young children was actually lowest in the exposed cohort, with only two cases arising in that group.

Although the incidence of all cancers combined was not increased among children in the exposed period compared with that among children in the unexposed period, the incidence data on ependymoma deserve careful review. The increase in ependymoma incidence among children aged 0–4 years in the exposed period compared with the incidence in the preceding unexposed period might suggest that the increase in incidence was associated with exposure to SV40-contaminated poliovirus vaccine (Table 2). However, incidence of ependymoma was relatively low in some years when SV40 contamination was present, and the highest incidence was observed in 1964, when most children were too young to have received SV40-contaminated vaccine (Fig. 2). The overall incidence pattern across calendar year was difficult to discern because of the paucity of cases. However, smoothing of the incidence data for children aged 0–4 years suggested a peak in incidence in 1969 (Fig. 2), well after the poliovirus vaccine was cleared of SV40 contamination. In addition, longer-term follow-up provided little evidence for elevated ependymoma incidence among SV40-exposed children (Table 1). On balance, these results do not suggest an effect of SV40 on ependymoma risk.

We used birth date to impute vaccination status, which we believe reliably classified Danish individuals with respect to SV40 exposure. The accuracy of this classification depends on two assumptions: that almost all children alive in Denmark from 1955 through 1962 were exposed to live SV40 and, to a lesser extent, that SV40 exposures ceased by 1963 following clean-up of the poliovirus vaccine. Regarding our first assumption, we have already described the highly efficient implementation of the Danish poliovirus vaccination campaign that began in the spring of 1955, which resulted in close to 100% vaccination coverage in the targeted age groups (17,18). In addition, we reviewed original internal documents at the Statens Serum Institut, which revealed compelling evidence that most, if not all, early lots of poliovirus vaccine contained live SV40. It seems likely, therefore, that most Danish children in the 1955–1962 era were exposed to live SV40. In comparison, estimates regarding the prevalence of vaccine contamination in the United States suggest that only 10%–30% of poliovirus vaccine lots contained live SV40 (1).

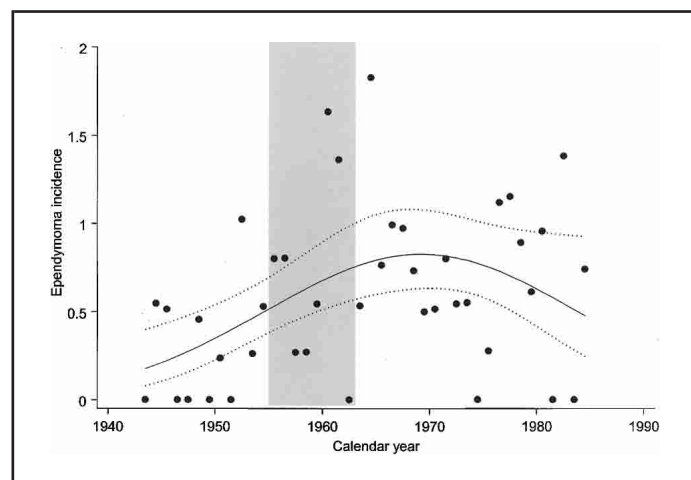Regarding our second assumption—that SV40 exposures

Fig. 2. Ependymoma incidence among children aged 0–4 years as a function of calendar year. The **shaded** time period corresponds to the years 1955 through 1962 when Danish poliovirus vaccine was contaminated with simian virus 40. Also shown is a smooth curve fitted to the data using Poisson regression (**solid line** = fitted values; **dotted line** = two-sided 95% confidence intervals). On the log scale on which this regression was conducted, this curve is a quadratic polynomial. On the *x*-axis, tick marks indicate the start of each year (i.e., January 1), and data points are centered over the middle of each year. Incidence is per 100 000 person-years.

ceased by 1963 following clean-up of the poliovirus vaccine—no epidemiologic study has been conducted to determine whether SV40 presently infects humans or is transmitted among asymptomatic individuals. Such studies have been difficult to conduct without serologic tests that have demonstrated sensitivity and specificity for SV40 infection. Routes of transmission for polyomaviruses, such as BK and JC viruses in humans and SV40 in macaques, are not well established. BK and JC viruses are frequently detected in human urine, and seroprevalence studies indicate that infections with these viruses occur early in life, perhaps through exposures to urine. Although SV40 can be similarly detected in macaque urine, data for humans are limited and conflicting *(29,30)*. Interestingly, BK and JC viruses are present in human sewage from Europe and South Africa, whereas SV40 is not *(31)*, suggesting that SV40 is not a common human infection. In any event, the circumstances for Danish children from 1955 through 1962 were unique in that SV40 exposure was ubiquitous and occurred early in life, when SV40 might be more likely to induce cancer *(19)*. If SV40 infections occurred after 1962, they would likely have been less frequent, have occurred at older ages, and have arisen from smaller inocula of virus. Thus, even without definitive evidence on whether SV40 currently infects humans, follow-up of vaccine-exposed children remains informative.

A unique strength of our study was the availability of high-quality nationwide data on cancer incidence in Denmark *(22)*, which go back to 1943. We considered that a possible limitation in our examination of data over such an extended period might be that countervailing changes in cancer incidence may have obscured an effect of SV40. Along these lines, incidence of all cancers combined, of all brain and nervous system tumors combined, of non-Hodgkin's lymphoma, and of testicular carcinoma increased gradually across birth cohorts (Table 1). Changes in registration are unlikely to explain this pattern. Although the Danish Cancer Registry's coding system has evolved over time, registration has been population-based and essentially complete over the entire period that we studied *(22)*. It remains unknown whether particular exposures might be responsible for these increases in cancer incidence or whether improvements in diagnostic techniques (e.g., radiologic imaging) played a role. However, modest increases in cancer incidence across birth cohorts or calendar year similar to the size observed in our study could not have hidden a large effect of SV40 if one was present.

Although the case numbers for some cancer outcomes were small in our study, our incidence data were of high quality and, for ependymoma and choroid plexus tumors, included only histologically verified cases. Using similar statistical methods, Strickler et al. *(32)* did not find increased cancer risk in U.S. cohorts exposed to SV40-contaminated inactivated poliovirus vaccine. Our study extends their results with incidence data on additional malignancies and on childhood cancers dating back to 1943. In Germany, risk of all central nervous system tumors, and specifically ependymoma, was not linked to receiving SV40-contaminated, oral poliovirus vaccine *(33)*. In addition, in a 35-year follow-up study of a cohort of 1073 neonates with documented exposure to SV40-contaminated poliovirus vaccines, only four cancer deaths were identified: two from leukemia and two from testicular carcinoma *(34)*. These retrospective follow-up studies do not suggest that exposures to SV40 increase cancer risk.

Our results and those of other retrospective studies *(32–34)*

thus conflict with laboratory reports of the presence of SV40 DNA sequences in human tumors, including mesothelioma, brain tumors, bone tumors, and non-Hodgkin's lymphoma *(9–15)*. Unfortunately, interpretation of the laboratory data is not straightforward *(4)*. SV40 DNA sequences are present in more than 200 cloning vectors used by laboratories worldwide *(35)*, which might conceivably lead to contamination of tumor tissues during laboratory evaluation. Indeed, the diversity of tumors in which SV40 DNA has been found and the low levels of SV40 DNA that are detected in these tumors [relatively few tumor cells harbor SV40 DNA *(4,36)*], raise suspicion that some findings could be artifactual. Some laboratories have not detected SV40 DNA in human tumors or have detected it infrequently *(37–43)*. In addition, SV40 DNA sequences were not reproducibly identified in a study in which mesothelioma specimens were evaluated blindly by multiple laboratories *(44)*.

In conclusion, our population-based, retrospective follow-up study does not support the hypothesis that SV40 is a cause of human cancer. Available epidemiologic data conflict with some, but not all, laboratory studies, indicating that further research is needed to settle this issue. Whether SV40 is transmitted among humans is a relevant question, and this question could be addressed by future epidemiologic studies that use serologic tests currently under development. Given the widespread exposure to SV40-contaminated vaccines in the 1950s and early 1960s, this issue remains of public health importance.

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